

[0013] In some embodiments of the antigen-binding constructs described herein, the first antigen-binding polypeptide construct comprises T30Q (H_T30Q), T30N (H_T30N), T30Y (H_T30Y), or T30F (H_T30F) in CDR1 of the VH domain. In some embodiments, the first antigen-binding construct comprises H_T30Q and H_K75W; H_T30Q and H_S74W; H_T30Q and H_S99W; H_T30Q and L_Y96G; H_T30Q and H_K75E; H_T30Q and H_G56Y; H_T30Q, H_K75W and L_Y49W; H_T30Q, H_K75W and H_S99W; H_T30Q, H_K75W and L_Y96G; H_T30Q, H_S99W and L_Y49W; H_T30Q, L_Y49W and L_Y96G; H_T30Q, H_S99W and L_Y96G; H_T30Q, H_G56Y and H_S99W; H_T30Q, H_K75W, H_S99W and L_Y49W; H_T30Q, H_K75W, L_Y49W and L_Y96G; H_T30Q, H_K75W, H_S99W and L_Y96G; H_T30Q, H_S99W, L_Y49W and L_Y96G; or H_T30Q, H_G56Y, H_S99W and L_Y49W; H_T30Q, H_G56Y, L_Y49W and L_Y96G.

[0014] In some embodiments of the antigen-binding constructs described herein, the first antigen-binding polypeptide construct comprises H_G56Y or H_G56F in CDR2 of the VH domain. In some embodiments, the first antigen-binding construct comprises H_G56Y and H_T30R; H_G56Y and H_K75W; H_T30Q, and H_G56Y; H_T30Q, H_G56Y and H_S99W; H_T30Q, H_G56Y, H_S99W and L_Y49W; or H_T30Q, H_G56Y, L_Y49W and L_Y96G.

[0015] In some embodiments of the antigen-binding constructs described herein, the first antigen-binding polypeptide construct comprises H_S99W. In some embodiments, the first antigen-binding polypeptide construct comprises H_K75W and H_S99W; H_T30Q and H_S99W; H_K75E and H_S99W; H_T30Y and H_S99W; H_K75W, H_S99W and L_Y49W; H_T30Q, H_K75W and H_S99W; H_T30Q, H_S99W and L_Y49W; H_T30Q, H_G56Y and H_S99W; H_T30Q, H_K75W, H_S99W and L_Y49W; or H_T30Q, H_G56Y, H_S99W and L_Y49W.

[0016] In some embodiments of the antigen-binding constructs described herein, the first antigen-binding polypeptide construct comprises L_Y96G. In some embodiments, the first antigen-binding construct comprises H_K75W and L_Y96G; L_Y49W and L_Y96G; H_T30Q and L_Y96G; H_K75W, L_Y49W and L_Y96G; H_T30Q, H_K75W and L_Y96G; H_T30Q, L_Y49W and L_Y96G; H_T30Q, H_K75W, L_Y49W and L_Y96G; H_T30Y, H_K75W, L_Y49W and L_Y96G; or H_T30Q, H_G56Y, L_Y49W and L_Y96G.

[0017] Also described herein are antigen-binding constructs comprising a first antigen-binding polypeptide construct that binds to ECD2 of HER2, wherein the antigen-binding polypeptide construct comprises one or more of CDR-H1; CDR-H2; CDR-H3, CDR-L1; CDR-L2; and CDR-L3 of variant 12536, variant 12514, variant 12491, variant 12490, variant 12482, variant 12480, variant 12479, variant 12478, variant 14042, variant 14057, variant 14058, variant 14060, variant 14032, variant 14035, variant 14062, variant 14041, variant 14044, variant 14051, variant 14055, variant 14045, variant 14047, variant 14056, variant 14059, or variant 14063. In some embodiments, the antigen-binding polypeptide construct comprises CDR-H1; CDR-H2; and CDR-H3 of variant 12536, variant 12514, variant 12491, variant 12490, variant 12482, variant 12480, variant 12479, variant 12478, variant 14042, variant 14057, variant 14058, variant 14060, variant 14032, variant 14035, variant 14062,

variant 14041, variant 14044, variant 14051, variant 14055, variant 14045, variant 14047, variant 14056, variant 14059, or variant 14063.

[0018] In some embodiments of the antigen-binding constructs described herein, the antigen-binding constructs further comprising a second antigen-binding polypeptide construct which specifically binds a second antigen, a first linker polypeptide operably linked to the first antigen-binding polypeptide, and a second linker polypeptide operably linked to the second antigen-binding polypeptide, optionally wherein the first and second linker polypeptides are capable of forming an interface with each other.

[0019] In some embodiments of the antigen-binding constructs described herein, the antigen-binding construct is monovalent and comprises a first antigen-binding polypeptide construct that is a Fab or an scFv, or the antigen-binding construct is bivalent and comprises a first antigen-binding polypeptide construct that is a Fab or an scFv, and a second antigen-binding polypeptide construct that is a Fab, an scFv, a single-chain Fab (scFab), a VHH, a domain antibody, or a peptide or polypeptide that binds to the second antigen, e.g., a HER2 ECD4 antigen or a HER2 ECD2. In some embodiments, the first and second linker polypeptide are capable of forming a covalent linkage with each other, optionally a disulfide linkage. In some embodiments, the first and second linker polypeptide each comprise an immunoglobulin hinge region from IgA, IgD, IgE, IgG, or IgM. In some embodiments, the first and second linker polypeptides are operably linked to a scaffold, e.g., an Fc, a human Fc, a human IgG Fc, or a human IgG1 Fc, e.g., a homodimeric Fc or a heterodimeric Fc. In some embodiments, the first and second linker polypeptides are operably linked to a heterodimeric Fc comprising first and second Fc polypeptides each comprising a CH3 sequence, wherein the first Fc polypeptide is operably linked to the first linker polypeptide, and the second Fc polypeptide is operably linked to the second linker polypeptide. In some embodiments, the CH3 sequence of each Fc polypeptide comprises one or more modifications that promote the formation of a heterodimeric Fc with stability comparable to a wild-type homodimeric Fc, e.g., the CH3 domain of the heterodimeric Fc has a melting temperature (T_m) of about 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 77.5, 78, 79, 80, 81, 82, 83, 84, or 85° C. or higher, as determined by DSC (differential scanning calorimetry).

[0020] In some embodiments of the antigen-binding constructs described herein, the heterodimeric Fc is formed with a purity greater than about 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% when expressed, optionally, when expressed via a single cell, as determined by LCMS (liquid chromatography mass spectrometry).

[0021] In some embodiments of the antigen-binding constructs described herein, they comprise a heterodimeric IgG1 Fc having the modifications L351Y_F405A_Y407V in the first Fc polypeptide, and the modifications T366L_K392M_T394W in the second polypeptide; a heterodimeric IgG1 Fc having the modifications L351Y_F405A_Y407V in the first Fc polypeptide, and the modifications T366L_K392L_T394W in the second Fc polypeptide; a heterodimeric IgG1 Fc having the modifications T350V_L351Y_F405A_Y407V in the first Fc polypeptide, and the modifications T350V_T366L_K392L_T394W in the second Fc polypeptide; a heterodimeric IgG1 Fc having the modifications T350V_L351Y_F405A_Y407V in the first Fc